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Note

Ultrastructural characterization and further transmission studies of *Thelohania solenopsae* from *Solenopsis invicta* pupae

Thelohania solenopsae is a microsporidium infecting several species of fire ants in the *Solenopsis saevissima* complex, including the imported fire ant species found in the US (Allen and Knell, 1980; Williams et al., 1998). Although the microsporidium was reported about 30 years ago (Allen and Buren, 1974), its mode of transmission remains obscure and ultrastructual studies are not complete. Four spore types (Table 1) have been reported: uninucleate meiospores within a sporophorous vesicle, two types of binucleate nonmembrane-bound free spores with different polar filament lengths (Knell and Allen, 1977; Sokolova and Fuxa, 2001), and binucleate nonmembrane-bound free megaspores (Sokolova and Fuxa, 2001). Recently, large numbers of previously unrecognized binucleate spores were reported from *Solenopsis invicta* pupae infected with *T. solenopsae* (Oi et al., 2001). The results of *T. solenopsae* transmission experiments by the same authors suggested that these spores in pupae might play a role in intracolony infection (Oi et al., 2001). Here we present an ultrastructural description of the binucleate spores from *T. solenopsae*-infected *S. invicta* pupae and results of additional transmission studies.

Pupae of *S. invicta* infected with *T. solenopsae* were from a polygyne colony collected 21 June, 2001 in Alachua County, Florida. Soft tissues of *T. solenopsae*-infected *S. invicta* pupae were dissected out in 2.5% (v/v) glutaraldehyde in 0.1 M cacodylate buffer containing 0.1% calcium chloride and fixed for 3 h in 2.5% glutaraldehyde solution containing 1% acrolein. Prior to fixation the glutaraldehyde/acrolein solution was warmed to 60 °C. Following fixation, samples were washed three times in 0.1 M cacodylate buffer (pH 7.2) and postfixed in 1% (w/v) aqueous osmium tetroxide for 1 h at room temperature. Samples were washed three times with water and dehydrated through a standard ethanol series (30–100% ethanol, 15 min per step), and then cleared with two washes in acetone. Tissues were infiltrated with Epon-Araldite resin. Sections were examined with a Hitachi H-600 electron microscope at an accelerating voltage of 75 kV.

All life cycle stages observed were diplokaryotic and in direct contact with the host cell cytoplasm. The spherical or ovoid binuceate meronts were limited by a simple plasmalemma (Figs. 1 and 2). These immature stages and mature spores were found tightly packed in the pupal fat body (Fig. 3). Germinated spores were not observed in any of the sectioned material. There was no interfacial envelope (sporophorous vesicle) present at any point in the developmental cycle. Merogony by binary fission produced areas containing numerous meronts (Fig. 3). The late stage meronts transformed into diplokaryotic sporonts characterized by a thickening of the plasmalemma and elongation of the cell (Fig. 4). Sporogony was disporous and sporogenesis began with the formation of the exospore and the primordium of the polar filament (Figs. 5 and 6). The ovoid binucleate spores were characterized by a short isofilar filament that made 3–4 coils around a large posterior vacuole, a bipartite lamellar polaroplast, and polyribosomes bordering the nuclei (Figs. 7 and 8). The spore wall was relatively thin and composed of three layers: an external unlayered electron dense exospore, a median lucent endospore of the same thickness, and an internal plasmalemma (Fig. 8).

A transmission test was conducted with the binucleate spores from pupae described above. Approximately 35 g of live, mostly non-melanized pupae were separated from a *T. solenopsae*-infected *S. invicta* colony. Pupae and water were macerated in a blender and then filtered through a cotton plugged syringe to remove debris. Filtrate was centrifuged at 4000 rpm for 3–4 min; the resulting pellet was reconstituted in water and centrifuged again at 10,000 rpm for 20 min. The final pellet was suspended in water (2 ml) and spore concentration was determined with a hemacytometer. Spores (1.28×10^7) were mixed into 1 g of hard-boiled chicken egg yolk. Jouvenaz et al. (1981) used an egg yolk formulation to successfully transmit the microsporidian *Burenella dimorpha* in the tropical fire ant, *Solenopsis geminata*. Eight, uninfected *S. invicta* colonies each consisting of 5 g (~6000 individuals) of worker caste adults, 5 g (~12 ml) of brood (eggs, larvae, and pupae) and the queen that founded the colony were starved for 4 days and then half of the colonies were given access to 1 g of the spore/egg yolk mixture for the duration of the study. The other four colonies were provided egg yolk only and ants were observed feeding on both the spore

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Table 1
Comparative characteristics of five spore types of *T. solenopsae*

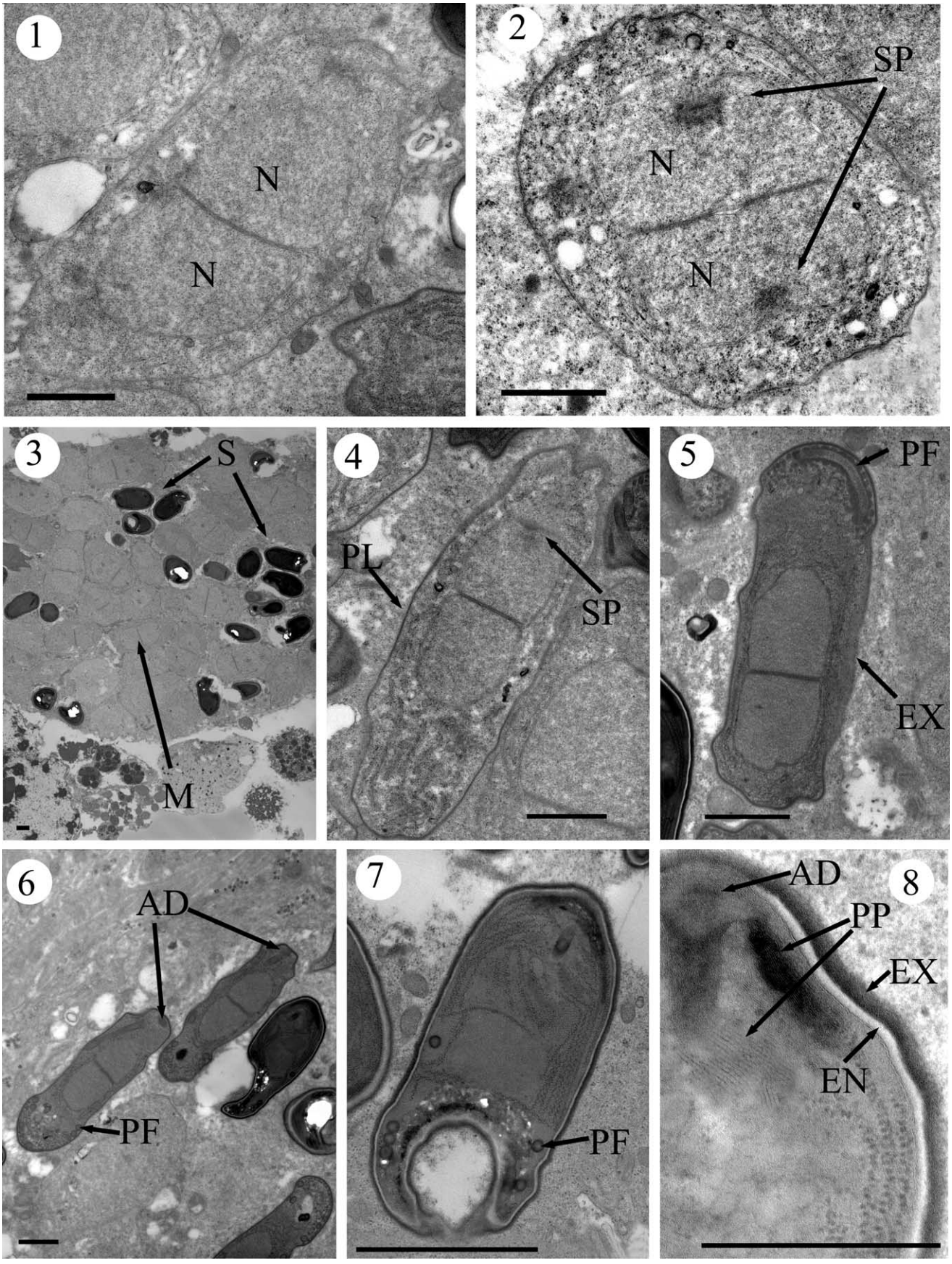
Spore type	Uninucleate meiospores	Binucleate spores	Binucleate spores	Binucleate spores	Binucleate megaspores
Reference	Knell and Allen (1977)	Knell and Allen (1977)	Present study	Sokolova and Fuxa (2001)	Sokolova and Fuxa (2001)
Length (μm)	3.32 ± 0.48	4.93 ± 0.58	4.5 ± 0.1	4.5 ± 0.29	7.2 ± 0.51
Width (μm)	1.95 ± 0.20	1.85 ± 0.16	2.3 ± 0.05	2.6 ± 0.23	3.8 ± 0.26
Host stage(s)	Pupae, adult workers, alates, queens	Adult workers	Pupae	Brood, adults of all castes	Brood, adults of all castes
Host tissue	Fat body, oocytes	Fat body	Fat body	Oocytes, fat body	Oocytes, fat body
Coils of polar filament	9–11	9–11	3–4	17–19	20–22
Sporophorous vesicle	Present	Absent	Absent	Absent	Absent

and control egg yolk formulations. After 5 days, a regular diet of frozen crickets and a sugar–water solution was provided to all colonies. No evidence of *T. solenopsae* infection was observed from any of the colonies based on wet mounts of adult workers sampled at 9, 14, and 19 weeks after initial access to spores, and giemsa-stained larval smears (Williams et al., 1999) sampled at 5, 9, 14, and 19 weeks (10 smears per sample). Queens died or larvae were not available in 1 treated and 2 control colonies, thus larval samples could only be obtained at 9 weeks from these colonies. Wet mounts of surviving queens were examined for infection when the study was terminated on week 20. Remaining treated ($n = 3$) and control ($n = 2$) colonies had average increases in brood of at least 356% (±368 SD) and 546% (±88 SD), respectively, at 19 weeks. Adults also increased 1067% (±601 SD) and 1717% (±684 SD), respectively. A similar test was conducted using 4.5×10^7 uninucleate meiospores, plus undetermined amounts of binucleate spores, that were isolated from adult workers using procedures described above. No evidence of infection was detected in either worker ants sampled 18 and 23 weeks after spore introductions, in larvae obtained on 4, 8, 16, 18, and 23 weeks, and queens on week 25. The treated ($n = 4$) and control ($n = 3$) colonies increased in brood 471% (±48 SD) and 237% (±285 SD), respectively, at 23 weeks. Similarly, workers increased 1903% (±281 SD) in the treated colonies and 1844% (±918 SD) in the controls.

This is the first ultrastructural description of *T. solenopsae* infections in *S. invicta* pupae. Knell and Allen (1977) reported vegetative stages in the pupae but did not provide descriptions of these stages. Ultrastructurally the spores were morphologically distinct from the other spores previously reported from *S. invicta* (Knell and Allen, 1977; Moser et al., 2000; Oi et al., 2001; Sokolova and Fuxa, 2001). This was the only spore type we observed in *S. invicta* pupae. Absence of germinated spores, restriction of the infection to the fat body, and the abundance of vegetative stages suggested that *T. solenopsae* was actively replicating in the pupal fat body but did not disseminate to other tissues This is consistent with the data on the restriction of *T. solenopsae* infection to the fat body of adults (Knell and Allen, 1977; Sokolova and Fuxa, 2001). The presence of meiospores in adults (Knell and Allen, 1977; Moser et al., 2000; Oi et al., 2001; Sokolova and Fuxa, 2001) is most probably a result of meiosis of the diplokaryotic cells carried over from the infected pupae. Reports of binucleate megaspores developing alongside meiospores suggest that some diplokaryotic mother sporont cells might undergo a different sporulation pathway or could simply represent aberrant forms.

Thus, five morphologically distinct *T. solenopsae* spore types have now been reported from *S. invicta* (Table 1). The most common spore type is the relatively small uninucleate meiospores contained within a sporophorous vesicle and produced in adult workers, alates, and queens. Additionally, there are three types of larger free binucleate spores of approximately the same size that differ in the length of the polar filament as well as by tissue/life stage specificity/caste. Finally, there are large free binucleate megaspores with long polar filaments. The free spores from adult worker fat body reported by (Knell and Allen, 1977), had 9–11 polar filament coils. The spores described in the present study

Figs. 1–8. Developmental stages of *Thelohania solenopsae* in *Solenopsis invicta* pupae. (1) Ovoid meront with two nuclei (N). (2) Spherical meront. Note the spindle plaques (SP) on the nuclear envelope in preparation for division. (3) Meronts (M) and spores (S) in the fat body. (4) Sporont characterized by a thickened plasmalemma (PL) and cell elongation. Note the spindle plaque (SP) on the nuclear envelope in preparation for division. (5) Early sporoblast characterized by formation of the exospore (EX) and primordium of the polar filament (PF). (6) Elongate sporoblasts with anchoring disk (AD), polar filament (PF) making 3–4 coils next to a mature spore. (7) Mature binucleate spore with polar filament making 3–4 coils and a large collapsed posterior vacuole. (8) Anchoring disk (AD), the bipartite lamellar polaroplast (PP) and cell wall showing unlayered exospore (EX) and endospore (EN). Bar = 1 μm.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EN

are restricted to pupal fat body, have 3–4 coils of the polar filament, and a large posterior vacuole. The *Nosema*-like spores described by Sokolova and Fuxa (2001) were found in adults of all castes (restricted to the fat body of workers and the ovaries of females), and had 17–19 polar filament coils. The megaspore described by the same authors had the same tissue/caste specificity (but rather larval than adult distribution) as the previous type and had a polar filament of 20–22 coils. The functional role of the five spore types described thus far from *T. solenopsae* has yet to be determined despite attempts in this and previous studies to initiate infections through transmission tests (Knell and Allen, 1977; Oi et al., 2001; Williams et al., 1999). Spore function remains a crucial objective in order to understand the horizontal and vertical transmission mechanisms involved in the spread and persistence of *T. solenopsae* in *S. invicta* populations in the US.

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References

Allen, G.E., Knell, J.D., 1980. Pathogens associated with the *Solenopsis saevissima* complex in South America. Proc. Tall Timbers Conf. Ecol. Animal Control Habitat Management 7, 7–94.

Allen, G.E., Buren, W.F., 1974. Microsporidian and fungal diseases of *Solenopsis invicta* Buren in Brazil. J. N. Y. Entomol. Soc. 82, 125–130.

Jouvenaz, D.P., Lofgren, C.S., Allen, G.E., 1981. Transmission and infectivity of spores of *Burenella dimorpha* (Microsporida: Burenellidae). J. Invertebr. Pathol. 37, 265–268.

Knell, J.D., Allen, G.E., 1977. Light and electron microscope study of *Thelohania solenopsae* n. sp. (Microsporidia: Protozoa) in red imported fire ant, *Solenopsis invicta*. J. Invertebr. Pathol. 29, 192–200.

Moser, B.A., Becnel, J.J., Williams, D.F., 2000. Morphological and molecular characterization of *Thelohania solenopsae* complex (Microsporidia: Thelohaniidae). J. Invertebr. Pathol. 75, 174–177.

Oi, D.H., Becnel, J.J., Williams, D.F., 2001. Evidence of intracolony transmision of *Thelohania solenopsae* (Microsporidia: Thelohaniidae) in red imported fire ants (Hymenoptera: Formicidae) and the first report of spores from pupae. J. Invertebr. Pathol. 78, 128–134.

Sokolova, U., Fuxa, J. 2001. Development of *Thelohania solenopsae* in red imported fire ants *Solenopsis invicta* from polygynous colonies results in formation of three spore types. In: Suppl. J. Eukar. Microbiol. Proc. Seventh Int. Worksh. On Opportunistic Protists, 85S.

Williams, D.F., Knue, G.J., Becnel, J.J., 1998. Discovery *of Thelohania solenopsae* from the red imported fire ants, *Solenopsis invicta*, in the US. J. Invertebr. Pathol. 71, 175–176.

Williams, D.F., Oi, D.H., Knue, J.G., 1999. Infection of red imported fire ant (Hymenoptera: Formicidae) colonies with entomopathogen *Thelohania solenopsae* (Microsporidia: Thelohaniidae). J. Econom. Entomol. 92, 830–836.

Alexandra M. Shapiro*
James J. Becnel
David H. Oi
David F. Williams
Center for Medical
Agricultural and Veterinary Entomology
USDA/ARS, P.O. Box 14565
Gainesville, FL 32604
USA
E-mail address: sshapiro@gainesville.usda.ufl.edu

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* Corresponding author. Fax: +352-374-5966.